14 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

14.1 Introduction:

14.1.1 Gas Chromatography/Mass Spectrometry (GC/MS) is a specific method of identification for most drug substances. MS can not differentiate between optical isomers. A sample is passed through a gas chromatographic column, effecting a separation of the components of the sample. The individual compounds then move into the mass spectrometer source where they are bombarded by electrons, producing charged ions. The ions of interest are positively charged fragments of the original compound. The ions are then separated, through a mass filtering process, according to their mass-to-charge ratios (m/z) and then collected by a detector. In the detector, the ion flux is converted to a proportional electrical current. The data system records the magnitude of these electrical signals as a function of m/z and converts this information into a mass spectrum. The mass spectrum is a record of the different ions (m/z) and the relative numbers of each ion (abundance). These spectra are characteristic for individual compounds, giving specificity for most types of drug substances.

14.1.2 There are three classical types of mass spectra which are well represented by different controlled substances.

14.1.2.1 The chemical structure of many controlled substances, such as methamphetamine and propoxyphene, is such that the base peak is quite large compared to other ions in the spectrum. The base peak generally represents greater than 50% of the total ion current contained in the spectra generated by these compounds. In drug compounds such as methamphetamine, the molecular ion may be relatively small in comparison to the $(M-1)^+$ ion. Other weak ions are important because they represent significant ionization pathways.

14.1.2.2 Cocaine and phencyclidine (PCP) provide a well defined mass spectrum with many peaks for comparison. These types of compounds generally present an electron impact (EI) mass spectrum in which the base peak represents roughly 30 - 50% of the total ion current generated by the compound. In this type of spectrum it is not unusual to see the base peak shift between 2-3 ions because of a limited number of highly stabilized ion fragments.

14.1.2.3 Heroin provides a well defined mass spectrum with many peaks for comparison. This spectrum is generated via multiple pathways resulting in many stable fragments of approximately equal intensity of about 10 -15% of the total ion current. In this type of spectrum it is not unusual to see the base peak shift between several ions because of the relatively large number of equally stable, low intensity ions generated by the EI process.

14.1.3 Although they are not considered a classical type of spectra, some drug compounds do not exhibit a molecular ion. Compounds that fit into this category include: barbiturates, lorazepam, and methylphenidate.

14.1.4 Confirmation of an unknown spectrum is done by direct comparison with a known or suitable reference spectrum or through use of classic interpretation methods. Positive mass spectral results may be recorded in the analytical notes by listing the drug identified. It is not required to record the analyst's disagreement with library search results on the data.

14.2 Procedure:

14.2.1 Samples will be dissolved in a suitable solvent, preferably methanol.

14.2.2 The general concentration should be determined by TLC or GC before being run on the GC/MS. The usual amount of sample delivered to the ion source for good qualitative results should be 8 - 160 ng. This correlates to an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio with a 1 µL injection volume. In any case, sufficient abundance of the total ion chromatogram peaks needs to be achieved in order to produce acceptable spectra, without overloading the chromatographic system.

14.2.3 Chromatographic conditions may be determined by the chemist from the GC/MS standards file.

14.2.4 The mass spectrum will be obtained in full scan mode using an appropriate scan range for the compounds to be analyzed.

14.2.5 At a minimum, a blank consisting of the solvent(s) used to dissolve the samples must be run on the GC/MS systems, when any of the following conditions are met:

- Before each analyst's series of sample runs whether manual or autosampler methods are utilized.
- No more than 10 samples can be run before another blank or standard/blank combination is required.
- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded or run between blank and sample.
- It is strongly suggested that a solvent blank be injected immediately prior to a sample known to be extremely weak.
- Additional blanks may be run at the examiner's discretion.

14.2.5.1 The solvent blank must be of at least as large an injection volume of the same solvent as the sample to be injected. The upper limit injection volume is normally 4 μL.

14.2.5.2 Any significant peaks in blank chromatograms must be properly investigated and documented in the referenced case file.

14.2.6 A background subtracted mass spectrum and normalized tabulation must be generated and included in the case file.

14.2.7 It is permissible to use GC/MS integrated retention times for GC retention time data. Standards used in the comparison must be run on the same day as the sample. "Same day" is defined as the approximate 24 hour period between autotunes.

14.2.8 Suitable comparison to in-house or reputable "library" spectra or published standard spectra should be done to verify sample identification. Due caution should be exercised when using the PBM similarity index generated by the library search algorithm. Spectra may also be identified through the use of classic interpretation methods in conjunction with data generated from additional testing.

14.3 Data Interpretation and Acceptance Criteria:

14.3.1 Integrated retention times for analytes are expected to agree with the standard within 2 seconds (± 2 sec.) or 0.03 minutes for this to be considered a positive GC result.

14.3.2 When barcodes are utilized for autosampler vial sample tracking, the barcode number should be printed on the data as well as documented in the case notes. If the barcode is not printed on the data during data analysis, it must be hand written and initialed after checking the vial's tray location.

14.3.3 In order for a mass spectrum to be considered definitive, all major peaks must have associated ^{13}C isotope peaks present.

14.3.4 For compounds such as cocaine, heroin and LSD, a molecular ion peak with associated ^{13}C isotope peak must be present in order for the result to be considered definitive.

14.3.5 For compounds, such as methamphetamine, amphetamine and related compounds, it is imperative that the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular ion be present in mass spectra in order for the result to be considered definitive. (e.g., methamphetamine must have a 148 and 149 *m/z* ion)

14.3.6 For compounds that do not exhibit a molecular ion, such as methylphenidate, the mass spectrum, when used in combination with TLC, retention time data and other testing, is sufficient for identification.

14.3.7 Some compounds should be derivatized to improve chromatographic performance or confirm the predicted molecular ion. Techniques of derivatization include silylation, alkylation and acetylation. Compounds that fit into this category include: barbiturates and lorazepam. Alternate ionization methods for mass spectrometry (e.g., positive or negative chemical ionization or LC/MS) can also be used via Instrument Support with the approval of the supervisor.

14.3.8 Anomalous mass peaks occurring above the molecular ion must be explained with data documentation in the case file. This may be accomplished using the ion reconstruct function of the ChemStation software. The easily recognizable common bleed peak, 207 *m/z*, occurring above the molecular ion may be labeled as such on the spectrum without further data documentation.

14.3.9 The strength of the sample/sensitivity of the instrument can be enhanced in the following ways:

- Up to 4 µL of solution may be injected.
- The sample can be concentrated and placed into an autosampler vial insert.
- The split can be lowered to 10:1 without ill effects.
- Splitless methods may be employed for samples containing small amounts of drugs including, for example, residues, LSD and fentanyl.

14.3.9.1 If the spectrum still does not meet the criteria, it should be reported as “Insufficient for Identification”.

14.3.10 Chromatographic and mass spectrometer conditions will be dependent on the currently used instrument and technology available. If there is any question as to either, consult with the primary operator of the instrument being utilized.

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